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Short-term storage stability of NaOCl solutions when combined with Dual Rinse HEDP

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Keywords: etidronate, HEDP, HEBP, root canal, sodium hypochlorite

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Abstract

Aim To assess the stability of NaOCl solutions when combined with a novel product for clinical use, Dual Rinse HEDP, which contains etidronate (1-hydroxyethane 1,1-diphosphonate).

Methodology Mixtures of NaOCl solutions with Dual Rinse HEDP were prepared so that they initially contained 5.0%, 2.5%, or 1.0% NaOCl, and always 9.0% of dissolved Dual Rinse HEDP powder per total weight. NaOCl solutions alone were used as controls. The stability of these solutions over 8 h was assessed in transparent borosilicate glass bottles at ambient temperature (23°C). Subsequently, the effects of heating (60°C) or storing the solutions at 5°C were studied in polypropylene syringes. NaOCl concentrations were measured by iodometric titration, i.e. free available chlorine contents. Experiments were performed in triplicates.

Results In the glass bottles at 23°C, the 5.0% NaOCl/9.0% Dual Rinse HEDP solution lost 20% of the available chlorine after 1 h, whilst the corresponding 2.5% NaOCl and 1.0% NaOCl solutions retained this relative amount of available chlorine for 2 and 4 h, respectively. Results obtained in the glass bottles were similar to those achieved in the syringes. Heating of the NaOCl/Dual Rinse HEDP mixtures had a detrimental effect on available chlorine, with a complete loss after 1 h. In contrast, storing the NaOCl/Dual Rinse HEDP mixtures in a refrigerator at 5°C kept the available chlorine high for 7 h, with the expected loss after a further hour of storage at 23°C.

Conclusions Initial NaOCl concentration and temperature both affected short-term storage stability of combined solutions containing Dual Rinse HEDP.

Introduction

Etidronic acid or 1-hydroxyethane 1,1-diphosphonic acid (HEDP or, less commonly, HEBP) is a nitrogen-free bisphosphonate used in water treatment, as a bar soap preservative, and in food disinfection (Mattia *et al.* 2006). An etidronate is a salt of etidronic acid, in which cations are bound to the anion of HEDP (usually Na_2HEDP or Na_4HEDP). Based on the unique short-term compatibility of HEDP with sodium hypochlorite (NaOCl), it is theoretically possible to use a combined NaOCl/HEDP irrigant during chemomechanical root canal preparation and for final irrigation (Zehnder et al. 2005, Lottanti *et al.* 2009). This concept of "continuous chelation" (Neelakantan *et al.* 2012) in the context of root canal irrigation has gained momentum in endodontic research over the recent years. The proteolytic/antibacterial effects of the NaOCl, which are based on the free available chlorine in the system, are maintained (Arias-Moliz *et al.* 2014). At the same time the HEDP, which is a calcium sequestering agent (chelator), prevents the accumulation of smear layer and hard tissue debris (Paque *et al.* 2012). Studies have shown that this single combined-solution irrigation concept can have favourable effects on the adhesion of various types of sealers to root dentine (De-Deus *et al.* 2008, Neelakantan *et al.* 2012, 2015b). Furthermore, by virtue of its effect on the smear layer, HEDP can enhance the disinfection efficacy of NaOCl in experimentally infected root canals (Neelakantan *et al.* 2015a) and dentinal tubules (Morago *et al.* 2016).

Most of the above-mentioned *in vitro* studies used fresh 1:1 mixtures of aqueous NaOCl and HEDP solutions at 5% and 18%, respectively, resulting in a combined 2.5% NaOCl/9% HEDP solution that was then used for the experiments. This was done in laboratory studies. However, a product to be used in clinics and clinical investigations (i.e. a registered medical device) based on this chemistry has not been available. A recent investigation has shown that the tetrasodium *salt* of HEDP (i.e. the etidronate) rather than a solution can simply be dissolved in the NaOCl solution of the clinician's preference, resulting in a combined NaOCl/HEDP irrigant that is relatively stable for 1 hour (Biel *et al.* 2017). This has led to the development of a CE-marked product (Dual Rinse® HEDP, Medcem, Weinfelden, Switzerland). However, because this product is new, no peer-reviewed studies have hitherto been conducted with it, and basic practical questions have not been addressed. The chair-side addition of etidronate powder into a NaOCl solution has the disadvantage that chemical reactions will occur over time, and could hamper the clinical performance of such a combined irrigant. On the other hand, the use of particulate etidronate has some advantages over using a solution when it comes to practicability for the dentist and storage/transportation issues for the manufacturer.

It was the goal of this study to test the compatibility of Dual Rinse HEDP powder with NaOCl solutions. Whilst NaOCl/HEDP mixtures are not stable for 24 h (Zehnder *et al.* 2005), it is unclear whether they remain useful for the course of a working day, so that larger amounts of the mixture could be prepared in the morning, possibly be stored in the refrigerator, and used throughout the day. Furthermore, some clinicians like to use pre-heated NaOCl solutions (Sirtes *et al.* 2005). The effects of these concepts on combined NaOCl/HEDP irrigants have not been investigated. Two possible clinical scenarios were followed in this study: NaOCl solutions containing dissolved Dual Rinse HEDP were stored in transparent borosilicate glass vials used in irrigation/instrumentation devices, or they were kept in common polypropylene syringes. Effects on free available chlorine in NaOCl solutions of three different strengths were studied. The Dual Rinse HEDP content in combined solutions was kept constant at 9.0 wt%.

Materials and methods

NaOCl Solutions

All NaOCl solutions were prepared from a 10% NaOCl stock solution (PanReac Applichem, Darmstadt, Germany, LOT 0001028549). Because hypochlorite solutions of different concentrations vary in their salt content and hence their specific weight (Zehnder *et al.* 2002), the concentrations of NaOCl solutions are presented as wt/total wt values in this communication. Wt/vol measures, as they are listed on some NaOCl brands, can be misleading because volume is not a parameter that is considered by the iodometric titration method, which is the gold standard to assess the capacity of NaOCl solutions (Frais *et al.* 2001). Solutions were checked for their content in available chlorine by this method (Vogel 1962), using a titration apparatus (665 Dosimat; Deutsche Metrohm GmbH & Co KG, Filderstadt, Germany) and a precision balance (Mettler AT 261 DeltaRange, Mettler Toledo, Greifensee, Switzerland). On each experimental day the NaOCl solutions were freshly mixed and adjusted to their desired concentration by dilution with ultrapure water.

Mixtures with Dual Rinse HEDP

NaOCl solutions were mixed with 0.90 g of Dual Rinse HEDP powder (Medcem), which corresponds to the mean content per capsule. To always get a 9.0 wt% Dual Rinse HEDP solution, 9.10 g of the NaOCl solutions were used for the combined test irrigants. To get to

5.0%, 2.5%, and 1.0% NaOCl in these mixtures, 5.5%, 2.75%, and 1.1% NaOCl stock solutions were prepared and used, respectively. To obtain the corresponding pure NaOCl control solutions, each of these stock solutions was mixed with 0.90 g of pure water.

Storage in glass bottles at ambient temperature

To get a basic idea on the mid-term effect of Dual Rinse HEDP on available chlorine in NaOCl solutions of different strengths, 9.0% Dual Rinse HEDP solutions were prepared that contained 1.0%, 2.5%, and 5.0% NaOCl. These mixtures and their pure NaOCl counterparts all had ambient temperature (23°C). They were placed into clear 500-mL borosilicate glass bottles (VWR, Radnor, Pennsylvania, USA) with polypropylene screw caps. These bottles are used, e.g. in the SAF system (ReDent Nova, Ra'anana, Israel). To simulate the clinical situation, a hole was drilled in the cap, and a silicone tube was immersed in the test or control solution. Bottles were stored under conditions commonly found in dental operatories (23°C, artificial light). Available chlorine was determined after 1 h, 2 h, 4 h, and 8 h.

Storage in polypropylene syringes at different temperatures

Dual Rinse HEDP was mixed in an inert vial in 10 mL of NaOCl solution for 2 minutes and then drawn back into a 10-mL polypropylene syringe with a Luer-Lock opening (Omnifix, B. Braun, Melsungen, Germany). This was done in the current study using the 3 strengths of NaOCl described above. In a first set of experiments, NaOCl of ambient temperature (23°C) was used. Temperature of solutions in the syringes was determined using a hand-held calibrated laser device (Thermo Hunter PT-3LF, Optex, Nionohama Otsu, Japan). Thirty-gauge irrigation tips (Perio/Endo Irrigation Needle, Kerr Hawe, Bioggio, Switzerland) were attached to the syringes with their lids on. The plunger closed the rear end of the syringe. Solutions were stored horizontally for 1 h at 23°C.

To test the impact of heat, syringes filled with test and control solutions at 23°C were mounted in a heating device for endodontic irrigants (Syringe Warmer, KeyDent, ADS, Vaterstetten, Germany) set to 60°C for 1 h.

To assess the impact of cooling NaOCl/Dual Rinse HEDP solutions to improve their short-term storage stability, NaOCl stock solutions taken from the refrigerator (5°C) were used, and test and control mixtures were prepared as described. These were then stored horizontally in polypropylene syringes in the refrigerator at 5°C for 7 h. Subsequently, a first set of solutions (n = 3 syringes per group) was assessed for their available chlorine content. A second subset of test and control solutions was then stored for an additional 1 h at 23°C, and the final content of available chlorine was assessed as described.

Data presentation

All the data presented here derived from triplicate experiments performed in separate vials. The current data derived from careful chemical measurements obtained under controlled conditions. Hence, there was minimal variance in the results, as is indicated by the low standard deviations. Mean values and standard deviations are presented. Based on the measurement error of the current method, NaOCl concentrations are presented to the first digit.

Results

In a first set of experiments, the stability of NaOCl solutions kept in glass bottles was tested over the course of one working day (8 h). These assessments revealed that the concentration of the NaOCl solution that is used to prepare the Dual Rinse HEDP combined irrigant impacts NaOCl mid-term stability (Fig. 1). The 5.0% NaOCl/9.0% Dual Rinse HEDP solution started to lose significant amounts of its available chlorine (i.e. 20%) already after 1 h, whilst the 2.5% NaOCl und 1.0% NaOCl solutions containing 9.0% Dual Rinse HEDP remained relatively stable for 2 and 4 h, respectively. The reaction kinetics were influenced by the NaOCl concentration over the whole course of the experiment, with the higher concentrations reacting faster (Fig.1).

The results obtained after 1 h in the glass vials at ambient temperature were similar to those obtained in the syringes after the same time. The only slight difference was observed for the 5.0% NaOCl/9.0% Dual Rinse HEDP solution, which retained 4.0 ± 0.0% NaOCl after 1 h, compared to 3.7 ± 0.1% NaOCl in the syringes. The pure NaOCl solutions kept all of their available chlorine at ambient temperature, whilst the counterparts containing Dual Rinse HEDP lost chlorine according to the NaOCl concentration (Table 1).

The temperature of the solutions when taken from the syringe warmer, averaged over all the NaOCl concentrations under investigation, was 61 ± 3°C for the test and 61 ± 2°C for the control solutions. This heating of the NaOCl/Dual Rinse HEDP mixtures had a detrimental effect on available chlorine, with a complete loss of that compound after 1 h, whilst the pure NaOCl control solutions kept all of their available chlorine (Table 1). In contrast, cool storing the NaOCl/Dual Rinse HEDP mixtures in a refrigerator kept the available chlorine almost constant for 7 h, especially for the solutions containing 1.0% and 2.5% NaOCl (Table 1). Subsequent storage of these solutions for 1 h at ambient temperature (23°C) led to final available chlorine values that were comparable to those of counterparts that were freshly mixed at ambient temperature and stored for 1 h.

Discussion

This study yielded potentially useful information on the effect of a newly available HEDP salt (Dual Rinse HEDP, Medcem) on the capacity of NaOCl solutions, as assessed by the determination of available chlorine. Results showed kinetics of the oxidation-reduction reaction between NaOCl (OCl^-) and the Dual Rinse HEDP depend on the concentration of the NaOCl, and the temperature the combined solution is stored at.

The current investigation is limited by the fact that it was a pure laboratory study. However, situations that can occur in clinics were simulated, and containers that are used to store and deliver sodium hypochlorite solutions in the operatory were used. It was assumed that the maximum time to irrigate a root canal system using a syringe is 1 h, whilst the working day of a dentist/endodontist is 8 h. This study focused on the available chlorine in test and control solutions, because that is the most appropeiate and easiest way to determine the oxidation capacity of sodium hypochlorite solutions (Vogel 1962).

HEDP has a successful tradition of usage in municipal water treatment, swimming pool stain and scale control, as a bar soap preservative, and in food disinfection (Mattia *et al.* 2006). In some of these applications HEDP is combined with an oxidizing disinfectant, mostly peracetic acid or sodium hypochlorite. However, the use of highly concentrated NaOCl solutions is unique to root canal irrigation, and hence, the known compatibility between HEDP and strong oxidizing agents such as NaOCl had to be assessed under concentrated conditions. The current results are congruent with those published using technical-grade Na_4HEDP (Biel *et al.* 2017). There is, however, one seeming contrast between the current findings and the results published by Biel and co-workers: the 5% NaOCl solution that was used in their study kept 86 ± 2% of the available chlorine after 1 h at room temperature when 9% HEDP was in the system. This compares to 74 ± 3% observed here. The explanation for this incongruence is that a 5.0% NaOCl solution was used in the former study, whilst a 5.5% solution was used here to prepare the mixtures. In the hope of making data presentation as clear as possible for the reader of the current communication, slightly higher concentrated NaOCl solutions were used in this study, so that the mixtures containing 9.0% of the Dual Rinse HEDP salt contained exactly 5.0%, 2.5%, and 1.0% NaOCl by weight. It would appear that above 5.0% NaOCl, solutions become too reactive to be used in combination with HEDP, unless they are used immediately and completely after mixing. Bleach solutions that are used in some countries to irrigate root canals assessed with the method described here may contain up to 6.4% NaOCl (wt/total wt), whilst corresponding solutions designated for the dental market are less than 5.3% NaOCl (Jungbluth *et al.* 2012). Based on the current results, the heating of NaOCl/Dual Rinse HEDP mixtures must be discouraged. Whilst it is not beneficial to heat calcium sequestering agents such as HEDP to increase their performance (Coons *et al.* 1987), heating of diluted NaOCl solutions is popular amongst some clinicians to increase efficacy in the root canal system and keep caustic effects low if the solution is extruded over the root canal terminus or leaking through the rubber dam (Sirtes *et al.* 2005). In agreement with the study by Sirtes and co-workers (2005), the current investigation found no short-term effects of heat on the stability of NaOCl solutions (Table 1). Furthermore, the solutions were stored in transparent syringes or glass bottles under artificial room light, which may also accelerate the degradation of NaOCl (Clarkson & Moule 1998), although the pure NaOCl solutions also remained stable for the course of the experiment. This was done to mimic the clinical situation. The effect of light on the NaOCl/Dual Rinse HEDP mixtures was not studied, but may be worth investigating.

When treated by high heat in an inert environment, HEDP decomposes to acetate and phosphonic acid (Hoffmann *et al.* 2012, Xia *et al.* 2014). However, in the alkaline environment and in the presence of the highly reactive OCl^- present in the current conditions, the following reaction is likely to occur

(1) $C_2H_8O_7P_2 + 2\ NaOCl + H_2O \rightarrow CH_3COOH + 2\ H_3PO_4 + 2\ NaCl$

The resulting acetic acid and phosphoric acid are buffered by sodium hydroxide (buffered system, not shown in eq. (1)). Next to eq. (1), sodium hypochlorite will most likely decompose by the two following reactions, which are enhanced by temperature and under the influence of light.

(2) $3\ NaOCl\ (aq) \rightarrow NaClO_3\ (aq) + 2\ NaCl\ (aq)$ (autoxidation)

(3) $2\ OCl^-\ (aq) \rightarrow 2\ Cl^-\ (aq) + O_2\ (g)$

Once these reactions gain momentum, the available chlorine (in form of OCl^-, HOCl, NaOCl) will invariably be lost. As the pH of the mixtures remains above 7, the evolution of chlorine gas is most unlikely (Biel *et al.* 2017).

Cooling of the NaOCl/Dual Rinse HEDP mixtures in the refrigerator showed some beneficial effects on the short-term stability of these combined irrigants, especially with the 1.0% and the 2.5% NaOCl solutions. This may be interesting for clinicians who prepare their irrigants in the morning of a working day and want to avoid the chair-side mixing procedure. However, under cooled conditions, NaOCl is less effective (The 1979). Clinically, however, this may be negligible, as in the spatial environment of the root canal system with its high specific surface, irrigants reach body temperature in a short period of time (Sonntag *et al.* 2017).

Future studies should assess clinical effects of Dual Rinse HEDP, such as its impact on the reduction of bacterial load by NaOCl solutions, and possible side effects such as post-operative pain.

Conclusion

This basic study on interactions between the Dual Rinse HEDP salt and NaOCl solutions showed that the concentration of the NaOCl in the combined solutions and temperature both affect short-term storage stability.

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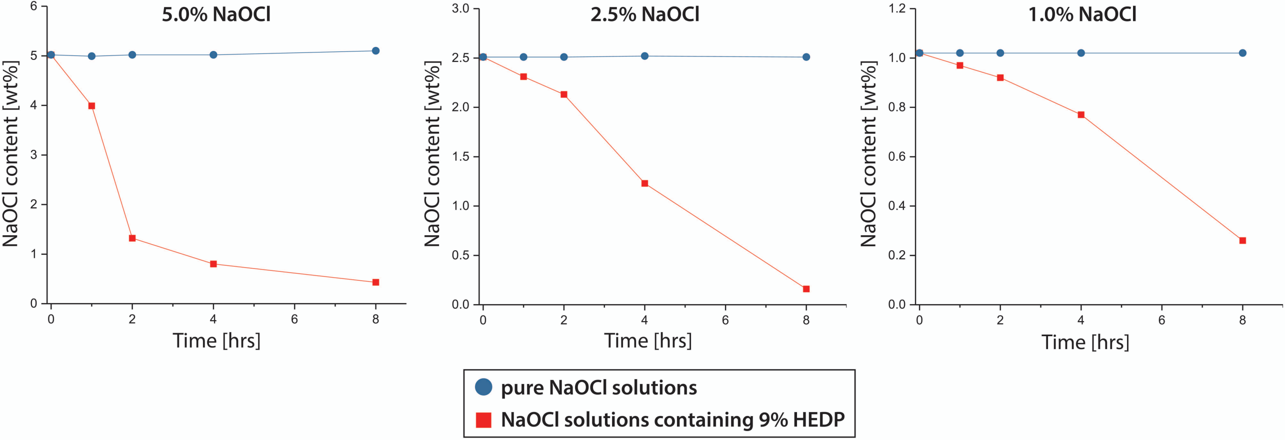
Caption

Figure 1 Evolution of NaOCl concentrations as assessed by iodometric titration in sodium hypochlorite solutions containing 9% of Dual Rinse HEDP per total weight. Solutions were stored in transparent borosilicate glass bottles at ambient temperature (23°C) and under artificial light.



5.0% NaOCl
2.5% NaOCl
1.0% NaOCl
NaOCl content [wt%]
Time [hrs]
pure NaOCl solutions
NaOCl solutions containing 9% HEDP

Table 1 Absolute wt/wt NaOCl concentrations, mean values (n = 3) and standard deviations, in different strengths* of NaOCl solutions in pure form (added water, Control) or with 9 wt% Dual Rinse HEDP (Test) according to the conditions they were prepared and stored in

Storage	Solution	1% NaOCl	2.5% NaOCl	5% NaOCl
23°C for 1 h	Control	1.0 ± 0.0%	2.5 ± 0.0%	5.0 ± 0.0%
	Test	0.9 ± 0.0%	2.3 ± 0.0%	3.7 ± 0.1%
60°C for 1h	Control	1.0 ± 0.0%	2.5 ± 0.0%	5.0 ± 0.0%
	Test	0.0 ± 0.0%	0.0 ± 0.0%	0.2% ± 0.0%
5°C for 7h	Control	1.0 ± 0.0%	2.5 ± 0.0%	5.0 ± 0.0%
	Test	1.0 ± 0.0%	2.4 ± 0.0%	4.1 ± 0.1%
5°C for 7 h, then 23°C for 1 h	Control	1.0 ± 0.0%	2.5 ± 0.0%	5.0 ± 0.0%
	Test	0.9 ± 0.0%	2.2 ± 0.0%	2.8 ± 0.2%

*Solutions were prepared from more concentrated stocks, i.e. 5.5%, 2.75%, and 1.1% NaOCl, so that they contained the presented amount of NaOCl when 0.90 g of Dual Rinse HEDP or water was added; they were stored in 10-mL polypropylene irrigation syringes enclosed by plunger and irrigation tip.

Note: the NaOCl solutions used for storage at ambient temperature or in the syringe warmer were warmed to ambient temperature (23°C) before the experiment, whilst the solutions to be kept cool were taken directly from the refrigerator (5°C).